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The Medico-Legal Significance of the Recently Discovered Henshaw (He) Blood Factor

In 1951 Ikin and Mourant[1] described a new blood factor in a Mr. Henshaw, a Nigerian. This was later shown by Chalmers *et al.*[2] to be closely associated with the MNS blood group system. They called the new factor Henshaw (**He**) and presented evidence that its relationship to the MNS system was, in its way, as intimate as that of the various factors comprising the Rh blood group system.

In this issue we publish a paper by Dr. M. Shapiro (Medical Director of the South African Blood Transfusion Service, Johannesburg) which supports the hypothesis advanced with more extensive family studies than have been published hitherto.

Interest in this new factor is considerable.

As far as is known, the **He** factor has no sensitizing properties in Man, i.e. it is of no importance clinically for blood transfusion. However, the close parallelism between the members of the extended MNS system and the complex Rh system opens up important genetic, forensic and anthropological applications which the new factor has made possible.

The existence of **He** has not yet been detected in Whites and its incidence is variable in negroids, e.g. 2·3% in Nigerians, 3·2% in American Negroes, 6·2% in South African Bantu and up to 14% in some Central African tribes. Anthropologically this is important, because it suggests that the **He** factor is predominantly a negroid trait; but even if it is eventually found in Whites, Asiatics or other ethnic groups, this fact alone will not necessarily indicate either recent or remote negroid admixture. In this respect, the position of the **He** factor is similar to that of the **V** factor (thought to belong to the Rh system) and the Hunter (**Hu**) factor of the MNS system.

Since rabbits are readily sensitized by the **He** antigen, the ease with which specific antisera can be prepared facilitates studies in connexion with the **He** factor and its widespread availability for forensic work is also ensured.

The main medico-legal importance of the **He** factor is illustrated by the following situations:

In Africa or the U.S.A., the detection of the **He** factor in freshly shed blood will, *prima facie*, suggest that the blood originates from a non-White.

1. Ikin, E. W. and Mourant, A. E. (1951): Brit. Med. J., **1**, 456.
2. Chalmers, J. N. M., Ikin, E. W. and Mourant, A. E. (1953): Brit. Med. J., **2**, 175.

In a community in which the **He** factor is present, there is a considerable extension of the chances of eliminating a falsely-accused father in a paternity dispute, in the same way as the finding of additional factors in the Rh system extended the usefulness of that system.

In the South African Bantu, the Rh system has a much more limited application in paternity studies than it has in Whites. The existence of this new factor (**He**) extends the value of the MNS system in negroid populations and compensates, to some extent, for the deficiency in the use to which the Rh system can be put amongst the Bantu.

As an individual factor, whose inheritance is determined in a simple Mendelian fashion, **He** has an immediate forensic application. But many more pedigree investigations of the type reported by Shapiro in this issue need to be completed before the gene combinations of the M-N-S-He system as a whole can have the same forensic force and validity as has the Rh system.

CORRESPONDENCE

Impartial Medical Testimony

To the Editor: Your editorial on *Impartial Medical Testimony* (**3**, 45-47, 1956) interested me for many reasons, not least of all because it recalled a Chapter in Lloyd Paul Stryker's *Courts and Doctors* (New York, The Macmillan Company, 1932). It is entitled *'What Constitutes an Expert'* and draws upon his paper in the *New York State Journal of Medicine* (**28**, 243-255, March 1928). In it Stryker records his objections to the court appointment of experts; but they may be applied, I think, to the establishment of a panel by medical societies as you have stated them.

Stryker also refers to a 1909 Committee of the New York State Bar Association acting together with State regular and homeopathic medical societies. The Committee's report offered 'the single but effectual method of defining to a jury with force and precision the distinction between a witness proven to be thoroughly qualified to speak upon the subject regarding which his testimony is offered, and one whose claim is predicated principally upon the fact that he is paid to do so. If trial judges will pursue this course and are sustained in so doing by the Appellate Bench, courts of justice will be rid of corrupt and worthless so-called experts, provided the judges are animated solely by the wish to see justice properly administered' (p. 146).

One point deserves mention which is sometimes overlooked. The doctor is really a consultant engaged by the attorney, with all the responsibilities shouldered by the latter. The lawyer, therefore, must decide with whom he can work best, the consultant's professional standing playing a prominent role, of course. It is not infrequent that satisfactory medical scientific status is not matched by understanding of the medicolegal problem posed in a lawsuit.

S. M. RABSON.

Coroner's Office,
Los Angeles 12,
California.
25 September 1956.

Blood Grouping Tests in Disputed Parentage

Qualifications of Experts

Alexander S. Wiener, M.D.

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The Committee on Mediocolegal Problems of the American Medical Association has recently published 2 reports dealing with the medicolegal application of blood grouping tests.[1, 2] In these reports the importance has been stressed of permitting only fully qualified experts to carry out blood tests in medicolegal cases, in order to avoid the possibility of errors and miscarriages of justice. With the growing appreciation of the value of blood grouping tests for establishing non-parentage, these tests are being applied more and more frequently, and the results of the tests are accepted more readily by courts. At the same time, with the increased demand for this type of examination, there has been a tendency for persons who are not fully qualified, to undertake performance of the tests. As a result, serious errors have been made. The present author had the opportunity recently to re-check the blood tests in a number of such cases, and uncovered errors which could have caused a serious miscarriage of justice. The purpose of this article is to present 3 of these cases, and to discuss what precautions should be taken to prevent similar errors in the future.

In blood transfusion practice it may be considered *a priori* evidence of negligence if a patient of group O is transfused with group A blood, or if an Rh-negative patient is given Rh-positive blood. If a patient dies from such a blood transfusion, the physician, the hospital and/or the blood bank may be subjected to a costly and unpleasant lawsuit for damages.[3] By the same token, an error in blood grouping in a medicolegal case of disputed parentage which falsely excludes paternity or maternity could conceivably lead to a lawsuit for malpractice and for damages for defamation of character. When performing blood tests in cases of disputed parentage, an error is even less excusable than in clinical cases, because the defense that the blood tests had to be done under pressure because of an emergency is not valid.

The medicolegal expert has plenty of time to carry out the tests, as many times as he desires and with as many controls as are necessary. If in doubt, he can always draw new blood samples and repeat the tests and in addition he can, if he wishes, send the blood samples to another expert for confirmation. Three of the leading workers in the field, all of whom have made important fundamental contributions to the subject and are certainly qualified to do these tests accurately, routinely send the blood specimens from their cases of exclusion of paternity to the author for confirmation. To date there has never been any discrepancy between the results obtained by these experts and by the author, demonstrating again how precise these tests can be in the hands of qualified persons.* Certainly, an inexperienced worker should have all his important reports confirmed by having the blood tests repeated by an expert, but there seems to be a direct relationship between the experience and knowledge of an investigator and the caution which he exerts. Thus, those workers whose reports are most in need of verification are the least sensitive to the possibility that their findings may be in error.

As a defense for a mistake, the fact that the reagents used were weak or nonspecific may not be used, because the qualified worker uses adequate controls and can readily ascertain the dependability of his reagents.† By the same token, if an error in blood grouping results from the use of faulty reagents, the laboratory which manufactured them cannot be held responsible—only the person who carried out the examination. There always exists the possibility that the serum may have deteriorated or become contaminated in the worker's own laboratory, and while the manufacturing laboratory may be expected to replace any serum which the purchaser finds unsatisfactory, it may not be held responsible for the user's mistakes.

Case Reports

Case 1. American citizenship is regarded as such a valuable prize that all sorts of plots and subterfuges have been used to gain admission to the U.S.A.[4] For example, many Chinese nationals have falsely claimed filial relationship to Chinese-American citizens in this country. To expose such frauds, the Department of Immigration adopted the policy that when applications for admission to this country are based on claims of filial relationship to citizens, the parties involved have to submit to blood group-

* Since the author is also subject to error, he welcomes confirmation of his results, especially when the findings are at all unusual. All reports of exclusion of paternity submitted to the Court of Special Sessions of New York City are routinely checked, thanks to the system set up by Corporation Counsels Sidney B. Schatkin and Sol Cooperman. Thus, the author's reports submitted to this court have been verified by independent tests carried out by another expert.

† Potent specific antisera for performing these examinations can be obtained from the Certified Blood Donor Service, Inc., Jamaica, New York, U.S.A.

ing tests. Unfortunately, no adequate provisions were made originally to be certain that the persons who performed the tests were fully qualified. The case to be described is one of the consequences of this near-sighted policy.

The author was consulted by an attorney regarding the following problem: The case involved a young man of Chinese extraction and an American citizen, who had been with the Armed Forces in Korea. While in Asia, he revisited his small home town in China, where he met and fell in love with a young woman. They married and had 2 children, and he then returned to the United States with his small family. When they reached Ellis Island, blood specimens were taken from him and his family for blood grouping tests. The specimens were tested by a technician in the laboratory of the United States Public Health Hospital at Ellis Island, who reported that the young man was not the father of the younger of the 2 children, because, according to his findings, the father belonged to type M, the mother to type N, the first child to type MN and the second child in question to type N.

The author then went to Ellis Island where he drew blood samples from the young man, his wife and the 2 children. The results of the author's examination are given in Table 1, which shows that the father belongs to

Table 1: Results of Blood Grouping Tests in Case 1

Blood of	*A–B–O Group*		*M–N Type*		*Rh–Hr Type*	
	Phenotype	*Genotype*	*Phenotype*	*Genotype*	*Phenotype*	*Genotype*
Father	O	*OO*	MN	*MN*	Rh_1rh	R^1r, R^1R^0, or R^0r'
Mother	A_1B	A_1B	N	*NN*	Rh_2Rh_0	R^1R^2, R^1r'', R^2r', R^2r, R^2R^0, or R^0r''
1st child ♀ 3 years old	A_1	A_1A_1, A_1A_2 or A_1O	MN	*MN*	Rh_1Rh_1	R^1R^1 or R^1r'
2nd child ♀ 1½ years old	B	*BB* or *BO*	N	*NN*	Rh_1rh	R^1r, R^1R^0, or R^0r'

type MN, and not to type M as originally reported, so that paternity is actually not excluded. In addition, paternity is not excluded by the A–B–O and Rh–Hr tests. In the Table, all the genotypes corresponding to each phenotype are listed, but by taking into account the family relationships, some of the genotypes can be eliminated as possibilities. For example, the 2 children obviously belong to genotypes A_1O and *BO*, respectively, since

the other 3 genotypes are excluded by the fact that the father belongs to group O. Similarly, since the first child belongs to type Rh_1Rh_1 she must have inherited an R^1 or r' gene from both parents, so that the 3 genotypes R^2r, R^2R^o, and R^or^y may be excluded as possibilities for the mother.

After the results of the author's blood tests were submitted to the Immigration Department, no further difficulties were placed in the way of the admission of the young man's family to this country.

The error in blood grouping in this case may be blamed on a common false notion. Not infrequently, in order to prove the reliability of blood examinations, the name of the institution where the tests were carried out is cited, since if an institution has an excellent reputation, the false inference is drawn that everything done there must be impeccable. The fallacy of course is that blood tests are carried out not by institutions but by natural persons, and it is the person who does the tests (and not the institution) who must be qualified. After having had experience with a number of such blunders in technique, the Immigration Department now permits only qualified persons from a list of experts provided by the local medical society to carry out these examinations.

Case 2. A couple who had a 9-year-old son had been divorced. The mother had remarried and was living with her second husband in New York City; the father had remarried and was living with his second wife in Milwaukee, Wisconsin, while the son was living with his maternal grandmother in Paris. The father had been contributing a small monthly sum in support of the son, until about 18 months ago when he discontinued the payments. The mother then instituted action to compel the father to resume payment, and to make good the back payments, but he counterclaimed by denying that he was the father and demanded blood tests.

The court in Wisconsin decided to have a physician from Milwaukee examine the blood of the putative father, while the present author was requested to test the mother's blood in New York, and a third physician in Paris to test the boy's blood. All reports were to be submitted to the physician in Wisconsin, who was to collate the results and submit his conclusions to the court. The blood of the mother was tested by the author in the usual way, and proved to belong to the groups A_1, M, Rh_1Rh_2.

Within a short time the present writer was surprised to receive a telephone call from the mother's second husband. In great distress, the latter stated that the physician in Wisconsin, comparing the results submitted by the physician in Paris on the child and the writer's results on the mother, had concluded that the blood tests showed that the woman was actually not the mother of her child, even though the question of maternity was not in issue. Upon hearing this, the putative father had seized upon this as evidence of fraud on the mother's part, and before long the entire story had appeared in the newspapers. The author then asked the mother's second

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husband for a copy of the report submitted by the physician, because it seemed highly probable that an error in technique had been committed. The exact wording of the physician's report was as follows:

The reports on the H matter are at hand. The blood of Mr. H was examined in this office. That of Mrs. H was examined by Dr. Alexander Wiener, Brooklyn, New York. The blood of Peter H was examined by Dr. X. at the —— in Paris, France. The following are the types reported:*

	AB System	*Rh (CDE) System*	*M–N System*
Mr. H.	O	Rh negative (c)	–MN–
	His cells gave negative reactions to anti-A, anti-B and anti-C, D, and E.		
Mrs. H.	A	Rh_1, Rh_2 (C, D, E, c, e)	–M–
	Her cells gave negative reactions to anti-B and Rh^w and anti-N serum.		
Peter H.	O	Rh positive (C, c, D, e)	–N–

Dr. X does not report the sera to which there was a negative reaction, but it can be reasonably assumed that they would correspond to the above findings.

Strangely enough these reports if taken on their face value would not necessarily rule out Mr H as the father but would rule out Mrs H as the mother. I am at a loss to interpret these findings since these tests have been made elsewhere. I would not care to pose as an interpreter of handwriting, but I have before me the stipulation sent by your office bearing the signature of Mrs H and Mr H. I also have the report of Dr Alexander Wiener who had Mrs. H sign the report and put a thumbprint on the record. All I can say is that the 2 signatures of Mrs H appear to be dissimilar but as to the accuracy of this I would defer to persons having greater knowledge of handwriting.

The exclusion is a clear-cut one in the MN factor. It is clearly understood that a pure N offspring must have the N factor in both parents. While the N factor does exist in Mr H's blood, according to our observation, it does not exist in Mrs H's blood according to the observation of Dr Wiener.

Therefore, I am unable to report on the matter of paternity but if these reports are to be taken at their face value I can report on the maternity and in this case there seems to be a question that this person who had the blood tests in New York is the parent of Peter H. This is certainly an unusual finding and I would be pleased to discuss it with you at any time.

The wording of this report supported the author's suspicion that an error had been made, and the author then instructed the mother to return to his office for a new examination. At the same time he instructed her second husband to have his attorney write to the physician in Paris to mail a sample of the child's blood to New York for testing. Re-examination of the mother's blood confirmed the original report; in particular, special tests excluded the possibility of the presence of the agglutinogen N_2. Thereupon, the following letter was written to the physician in Wisconsin while awaiting the

* This report becomes more intelligible when the results are tabulated as follows:

Blood of	*A–B–O Group*	*M–N Type*	*Rh–Hr Type*
Mr. H. (putative father)	O	MN	rh
Mrs. H. (mother)	A	M	Rh_1Rh_2
Son	O	N	Rh_1rh

blood specimen from the boy:

I was very interested to see the copy of your letter to Judge Y on the H case. Mrs H returned to my office and her blood has been retyped, with the identical results, of course. On Monday 2 April she will return again, and a specimen will be taken to be sent to you so that you can test her blood yourself. I am looking forward to receiving some of Peter H's blood from Paris and Mr H's blood from you so that I can test their bloods also.

I strongly suspect that Dr X has made an error in blood typing. The reason for this is that his book on blood groups is full of mistakes and misinformation, and the only two pages which are correct are translated from my own book without suitable acknowledgement. Moreover, anybody who cannot master the correct Rh–Hr terminology and uses the C–D–E symbols is obviously not qualified. A report on this question will appear in three or four weeks in the J.A.M.A.

Shortly after this letter was mailed the blood specimen of the child arrived from Paris. As expected, it was found that indeed an error had been made, since the child belonged to type MN and not N as originally reported. The following is the 'explanation' submitted by the Paris physician to account for his mistake:

I sent you, a few days ago, a sample of Peter H's blood, son of Mrs H We made the examination of this blood on 8 March. Two samples were taken by myself and separately studied in my Laboratory and the Dr M (Chief of Laboratory at the ——, Paris) one. In all, 6 different anti-M sera were used. Only one of them gave us a very slow agglutination. As we had sent the results quickly,* we could not ask for a new sample. Two days ago, we checked 2 new samples of this blood and even with the same anti-M sera we got strong agglutination. So, we agree with the following determination: O, MN Rh+ (CcDee). At the time of the first determination, the boy seemed to be in good health. Tubes and seringe (*sic*) used were sterilized and test cells of known donnor (*sic*) gave us good results. The examination was done a few hours after. I will make another exam of this blood on 15 April.

The original order for the blood tests in this case had been submitted to by the mother under a stipulation signed by her and the father in which it was agreed that the results of the tests would be accepted by both parties as final in deciding their case. Now that the mistake made by the expert in Paris had been corrected, making it clear that the blood tests excluded neither maternity nor paternity, one might expect that the father would have been satisfied. Instead, however, he continued to deny paternity, insisting that the confusion caused by the blood tests carried out by the Parisian expert rendered the original stipulation null and void. As a result, the case now had to be tried in open court.

It not infrequently happens that the parties involved in a case of disputed paternity live in different parts of the world. In such instances, it has been customary to have the blood from each person tested by a different expert, selected for geographic convenience. The separate reports of the different experts are collected by the court and compared, and a decision then rendered. For example, the author has not infrequently been requested to examine blood of persons involved in paternity disputes to be tried in courts in Denmark, Germany, France and other parts of the world. Since, with the advent of air mail, it is possible to send blood from any part of the

* So quickly that there was time to do the test only 18 times, from the description.

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world so that it arrives at its destination within one or two days, and in excellent condition, it would seem more prudent to send all the blood specimens to a single qualified worker so that he can test all the specimens, compare the results and render a report. This case also shows that the mere fact that a worker has published articles or even books on the subject, does not necessarily indicate that he is qualified to carry out blood grouping tests in medicolegal cases. Not every article on blood grouping that has been published in the medical literature has been written by a competent worker. In fact, the prevailing confusion regarding Rh–Hr nomenclature is largely due to the publication of many articles by persons who do not understand the subject thoroughly.[5] Fortunately, authoritative treatises on the subject are available both for specialists[6] and attorneys.[7]

Case 3. We were consulted by a physician from ——, Pa., because one of his patients had given birth to an illegitimate child and was involved in a court procedure. The man, who she stated was the father of her baby denied the charge and requested a blood test. This examination was carried out under the direction of the court by a local clinical pathologist, who had had no special training in the field of immunohematology. When his report excluded paternity, the mother insisted that this was impossible, since she knew that the defendant was the father. Her family physician was convinced that his patient was telling the truth, and sent her and the baby to the author in New York City, to have their blood retested. She brought with her the original report submitted by the physician designated by the court, which read as follows:

> In accord with your court order, I have performed blood tests on D. K., Baby K, and J H. I personally drew the blood samples at the ——, —, Pa., on Thursday, 12 April, 1956. The identification of these individuals was confirmed by acknowledgement, before me, of each by the other. The tests were performed by me personally using active fresh sera obtained from recognized laboratories. My findings are as follows:*
>
> Baby K.: Group O—C neg., D pos., E pos., c pos., M pos., N neg.
> D. K.: Group O—C neg., D pos., E pos., c pos., M pos., N neg.
> J. H.: Group O—C pos., D pos., E neg., c neg., M pos., N pos.
>
> On the basis of these findings, it is determined that with respect to the blood factor C (rh'), John H is homozygous his genotype being C-C, meaning that he must transmit this factor to any of his offspring. However, Baby K is of the genotype c-c, indicating the absence of the blood factor C. For this reason, J H. can be excluded as the father of this child.

From the nature of the report, it was apparent that the person who had carried out the original tests was not fully qualified. The report suggested

* These results become more intelligible when interpreted as follows:

Blood of	*A–B–O Group*	*M–N Type*	*Rh–Hr Type*
J. H. (putative father)	O	MN	Rh_1Rh_1
D. K. (mother)	O	M	Rh_2
Baby	O	M	Rh_2

that this was one of his first experiences with medicolegal blood tests, and that the antisera used had been purchased specially to be used for testing the persons involved in this case. The report assumed the 'simplest' possible form, namely, by indicating which antisera gave positive reactions and which did not, such as would be done by a neophyte who lacked knowledge and understanding of the fundamentals of blood grouping.

Accordingly, the author predicted to the referring physician that it was highly probable that an error in blood grouping had been made. Indeed, when the tests on the mother and her child were repeated, the following results were obtained:

Blood of	*Group*	*M–N Type*	*Rh–Hr Type*
D. K.	O	M	Rh_2
Baby	O	M	Rh_1Rh_2

Thus, an error in Rh–Hr typing had been made, since the child actually belonged to type Rh_1Rh_2 instead of type Rh_2. Obviously, therefore, the conclusion in the original report that paternity was excluded was likewise in error. Upon obtaining these results the author suggested to the attorney representing the woman to have the blood tests repeated by the expert who had carried out the original examination, in order to give him a chance to rectify his error. Instead, the attorney requested the author to go to ——, Pa., to testify.

The case was tried before a bench of 3 judges and a jury of 12 citizens. The woman testified first and gave a straightforward story indicating that the only man with whom she had had relations was the defendant. This testimony was not challenged by the defense, who instead placed their medicolegal expert on the stand. This witness made a fine appearance, and his qualifications were challenged unsuccessfully by the woman's attorney. With the aid of a blackboard he explained the results of the Rh–Hr tests, using both the Rh–Hr and C–D–E notations, and gave a most convincing demonstration that the defendant was not the father of the plaintiff's child. (Apparently the witness had studied up on the subject in preparation for his testimony.) The judges, however, had been informed that the author was in the court room and had obtained discrepant results, and after the witness had finished his testimony called the author before the bench. The author suggested that he and the local expert go to the latter's laboratory and repeat the blood tests on fresh samples of blood, in order to resolve the difference in the results. This was done, and before the end of the day it was possible to show, using the same reagents that the local expert had used originally, that the baby's blood indeed contained the **rh′** factor although this had been missed in the original test. In the meantime, attention was called to the fact that whereas the local expert had reported the

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putative father's blood group to be group O, the latter had donated his blood to several blood banks and possessed an identification card indicating that he actually belonged to group A. This additional discrepancy the local expert blamed on a typographical error in his report. The next morning, the local expert testified once more, and explained to the jury that he had made an error in Rh–Hr typing, so that the blood tests did not exclude the possibility that the defendant could be the father of the child after all. Thereupon, the judge instructed the jury to disregard this expert's previous testimony, and a verdict was then rendered in favor of the plaintiff.

Discussion

When blood grouping tests were originally introduced into use in cases of disputed paternity in New York City, no restriction was placed by the court on the selection of experts to carry out the examinations. Therefore, at first, many attorneys selected any physician with whom they were acquainted who stated he knew how to carry out blood tests, and a number of gross blunders resulted. One physician testified that an accused man belonged to group O International, group I Jansky, and group IV Moss, while the child belonged to group A International, group II Jansky, and group II Moss, and that therefore paternity was excluded. Cross-examination disclosed the fact that this 'expert' had performed only 3 or 4 blood grouping tests in his entire experience of 20 years. Apparently, the witness thought that by using all 3 nomenclatures his testimony would appear more learned, and impress the court. When asked why he had not tested the blood of the mother, he responded that the question of maternity was not an issue.

In a second case, a pathologist reported the putative father to be group B, the mother to be group O, and the child to be group A, so that paternity was excluded. Retests by the author showed the putative father to belong to subgroup A_2B and the child to subgroup A_2. As a result of this kind of experience, courts in New York City now permit the use only of experts whose names are included in a list provided by the New York Academy of Medicine.*

A fully qualified expert in blood grouping is not merely an accurate technician but a person thoroughly versed in the fundamentals of blood group serology and genetics. The facts which have been unearthed about the Rh–Hr types are exceedingly complex, but to experts these complexities offer no difficulty. Since the terminology merely translates the facts into symbols, to a person who thoroughly understands the subject, the Rh–Hr terminology offers no problem, and such a worker has no difficulty in preparing a clear self-explanatory report when presenting his evidence

* A more complete list of experts for the U.S.A. can be obtained by writing to the Law Department of the American Medical Association.

before a lay bench and jury. It is evident, therefore, that anyone who finds it necessary to resort to the incorrect C-D-E notations because this terminology is 'simpler' does not understand the subject thoroughly,[5] so that this may be used as one of the criteria by which to judge the qualifications of an expert. When applying this criterion, however, one must bear in mind that this rule has its exceptions.

Summary

Three cases are discussed in which errors in blood grouping were made in medicolegal cases of disputed paternity.

These experiences demonstrate how important it is that the person charged with the responsibility of carrying out blood grouping tests in cases of disputed paternity be fully qualified.

Such experts must not merely be accurate technicians, but also thoroughly versed in the basic principles of blood group serology and genetics.

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The Paranoid Murderer

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New York, U.S.A.

In a frank mental illness like schizophrenia, murder is often part of the psychic mechanism. Murder in this case serves as a definite defense against the ego-disintegrating effects of schizophrenia. One purpose of murder is to discharge overwhelming rage in persons threatened by mental collapse. There is a great deal of evidence that feelings of rage play an important part in the psychic economy of the schizophrenic. Quite often this rage seems to stem from frustrations encountered during early years. If, as in the potential schizophrenic, hatred is abundant and not discharged, it accumulates, leading to progressive isolation and alienation of the individual from his fellows and, through projection, to an increase in the number of enemies, antagonists and the malevolent forces arrayed against him. If the ego is not destroyed, the hatred must somehow be discharged.

The schizophrenic paranoid individual is more than ordinarily isolated from people. He feels insecure in the world of everyday reality and he protects himself from this insecurity by increasing the distance (physically or emotionally, or both) between himself and others. The paranoid overcompensates for his actual insecurity and paucity of experience or achievement by ideas of grandeur and omnipotence of varying degrees of distortion.

The paranoid potential murderer lives in a world of unrealistic self-importance. When his isolation is threatened by others' attempts to come close to him or by circumstances which interfere with his system for maintaining self-esteem, the paranoid's tension mounts to the breaking point.

Strangely enough, it is rare for this isolation to be entirely successful. The paranoid's repressed and suppressed wishes for affection and proximity to others, unconsciously carries him again and again into situations where he might find satisfying relationships if he could only accept them.

The paranoid may be driven to protection by means of violence of another person. He projects the feeling of persecution to this other person to whose aid he must go. For example, a man may complain that his wife's reputation is being ruined by malicious persons; he then decides to discharge his resentment against the fancied wrong by committing homicide.

In schizophrenia of acute onset the murder is usually preceded by a

period of unbearable tension and extreme anxiety associated with depression or agitation, in which case presumably unassuagable anger plays a leading and dominant role. In cases where the onset is gradual and insidious, the individuals may be passive types who offer little resistance to the disease, and their ego undergoes a slow disintegration. The more active types struggle with the disease and resist its onset as long as their ego-strength will permit. When the breaking point is finally reached, they may either give up the struggle or make a desperate last-minute attempt to ward off the psychosis. Quite a few commit murder when this occurs. This is so because only an aggressive act of great magnitude will suffice, the choice depending on the relative strength of inhibitions, social sense, etc.

Quite often the paranoid individual may be regarded as a person whose overgrown ego has led him to attempt what is for him an impossible achievement, and who has disowned his failure and preserved his self-esteem by building up a system of delusions. He welcomes punishment because it serves to strengthen his delusions. For the paranoid the ideal crime is one which brings him notoriety and martyrdom. This is in harmony with his delusions and is justifiable if his beliefs are true.

The schizophrenic process is a regressive adaptation in which the individual attempts to cope with overwhelming anxiety, arising when abhorrent cravings erupt into awareness. This eruption may precipitate a state in which cultural accretions are lost, primitive patterns gain sway, and the individual is concerned only with survival. Delusions, hallucinatory phenomena and ideas of reference may be used to create a state of omnipotence and thus cope with these feelings. When these fail, the only primitive pattern which will ameliorate the terror is intense motor activity culminating in direct aggression, such as murder, against a representative of a past significant figure. This figure may be a law officer, a judge, an employer, etc., or some one who bears a physical or symbolic resemblance to them. By this method the barrier to omnipotence is eliminated, and the significant figure symbolically destroyed.

The commission of a murder that lacks an adequate or comprehensible motive is a species of behavior that has intrigued students of mental phenomena for many years. The motiveless homicide is motiveless on the surface only. There are reasons for every human action, though these reasons may not be quite apparent at first glance.

The motiveless murder has as its basis an attempt to regain homeostasis in the internal milieu. There are several reasons for this disruption. One prominent reason for the motiveless murder is a defense against the ego-disintegrating effects of schizophrenia.

Thus, one purpose of the motiveless murder is to discharge intolerable and overwhelming rage in non-psychotic ambulatory schizophrenia threatened by a psychotic decompensation. It has definitely been established

that feelings of rage play an important part in the psychic constitution of schizophrenics. Schizophrenics may do violence to themselves or to others while in the grip of an acute psychotic decompensation. These destructive outbursts of rage are usually preciptated or accompanied by delusions and/or hallucinations appropriate to the individual's destructive and assaultive conduct.

Thus murders which lack an adequate motive may represent an attempted defense against the outbreak of a schizophrenic psychosis, in which the ego seeks to protect itself from disintegration by discharging intolerable anger through an act of extreme violence. In this way the physical act of murder may decrease the danger of psychic injury represented by the schizophrenic process.

The urgently immediate problem of the acutely disturbed individual, seeking an adequate defense against the outbreak of schizophrenia, is to discharge an unbearable and overwhelming amount of aggression, and only an aggressive act of tremendous magnitude can suffice for this purpose. When there is insufficient inhibition or when the sense of social responsibility is thwarted, the individual seeks release from this terrible inner drive by committing murder. Thus the primary purpose of motiveless murders is an attempt to obtain release from unbearable internal tension.

There are several quite distinct factors in cases of motiveless murders. These are personality changes which include shyness, withdrawal and attacks of melancholia. This is invariably followed by a state of extreme tension; then either an 'inner voice' or a feeling of extreme compulsion urges the individual to commit the act. The act itself is followed by lack of remorse and a state of calm.

That the primary purpose of the motiveless murder is to discharge pent-up hostility is indicated by the fact that most of them are characterized by unnecessary ferocity, which goes far beyond what would be required merely to kill the victim. The bloodiness of the murders, involving innumerable blows or stab wounds, is quite characteristic.

Another feature is the senselessness of the murder. The victim has done the murderer no harm; he is entirely unknown to him. He has become the victim as a result of the mechanisms of displacement of hostility from the real object, which (for various reasons) may not be consciously known and recognized as such by the murderer. Most often the murderer is strikingly calm after the act, and there is no expression of remorse. The fact that the murderer is calm is a good indication that the murder afforded him a release from intolerable tension.

Inheritance of the Henshaw (**He**) Blood Factor *

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In 1951 Ikin and Mourant[1] described an anomalous anti-**M** serum which agglutinated the blood of two group N Nigerians. The authors were first under the impression that they had re-discovered an antibody for the Hunter (**Hu**) factor described by Landsteiner, Strutton and Chase[2] in 1934. Chalmers, Ikin and Mourant[3] used the blood of one of the Nigerians, a Mr. Henshaw (who was group N) to immunize a group of rabbits. After removal of the anti-**N** component from the immune rabbit antisera, all were found to contain an additional antibody which agglutinated Henshaw's cells. One thousand five hundred Europeans were tested for the new blood factor and none was positive, but of 1,428 West Africans tested 33 (3·2%) were found to possess the factor. As a control, 138 West Africans were also tested with some of the original anti-**Hu** serum and 30 (21·7%) were found to react, thus demonstrating that the two factors were different from each other. The bloods reacting positively with anti-**He** and anti-**Hu** were fully phenotyped. Statistical analysis of the results disclosed that both factors were associated with the M-N-S blood group system, the **He** factor being associated almost exclusively with **N** and **S** factors in the phenotypes and the **Hu** factor with **N** and **s**.

Chalmers and his co-workers[3] also investigated the inheritance of the **He** and **Hu** factors in 10 families and came to the conclusion that their findings were consistent with complete linkage between the Henshaw (*He*) gene and *NS* or *Ns* and between the Hunter (*Hu*) gene and *Ns* or (each in one family only) *NS* or *Ms*. Among their pedigrees was a number of persons who gave unexpected results, but these they attributed to erroneous information regarding family relationships. These authors published only two family trees in substantiation of their hypothesis.

The present communication records the results of more extensive studies in South Africa. The distribution of the **He** factor has been investigated in 4 ethnic groups—Whites, Bantu, Malays and Cape Coloureds. These, together with the findings in Hottentots and Bushmen previously reported

* A paper read at the Annual Congress of the American Society of Human Genetics held at Storrs, Conn, U.S.A., in August 1956.

by Zoutendyk,[4] provide a fairly comprehensive picture of the ethnological distribution of the **He** factor in South Africa.

Material for inheritance studies was obtained by tracing the pedigrees of **He**-positive persons found in the Coloured sample. The detribalized urban Bantu are quite unsuitable for kindred studies because of the exceedingly high incidence of illegitimacy.

Unfortunately, it was not possible to include the **Hu** factor in these investigations. Unlike **He**, **Hu** is a poor antigen and although a large number of rabbits was inoculated in our laboratories with known **Hu**-positive bloods, none produced the antibody. However, Dr. Mourant kindly tested the bloods of 30 Bantu males on our staff and found 8 positives. From this it would appear that the frequency of **Hu** in South African Bantu is comparable with that of West Africans.

The distribution of **He** has so far been studied in only a few populations. Such information as is available suggests that the factor is essentially a negroid trait. Table 1 summarizes the published data for Africa. The sample of American negroes studied by Pollitzer has been included for purposes of comparison.

Table 1: Distribution of Henshaw (**He**) Factor in Various Populations

	Racial Group	M.S	M.s	MN.S	MN.s	N.S	N.s	*Totals*	%
South Africa	Whites[1] ..	0	0	0	0	0	0	0/1000	0
	Cape Malay[1] ..	0	1	1	0	0	0	2/107	2·0
	Cape Coloured[1]	5	22	5	14	0	1	47/1000	4·7
	Bantu[1] ..	20	70	18	101	6	32	247/4000	6·2
	Bushmen[2] ..	0	0	2	0	2	1	5/188	2·7
	Hottentots[2] ..	7	1	10	2	1	0	21/201	10·5
East Africa	Hira[3]	0	0	0	0	3	1	4/117	3·5
	Iraqw[3] ..	3	0	3	2	1	2	11/93	11·8
West Africa	Congolese[4] ..	3	1	4	1	0	1	10/70	14·0
	Nigerians[5] ..	0	0	15	0	17	1	33/1428	2·3
America	New York Negroes[6]	2	1	9	3	11	6	32/1000	3·2

1. Shapiro, M. 2. Zoutendyk, A. *et al.*[4] 3. Allison, A. C. *et al.*[9]
4. Nijenhuis, L.[6] 5. Chalmers, J. N. M. *et al.*[3] 6. Pollitzer, W. S.[10]

As in Europeans, **He** was not found in the South African Whites studied. The Congolese, Hottentots and Iraqw have the highest frequencies so far recorded. In all three, **He** appears to be predominantly associated with **M** and **S** in the phenotypes, in contra-distinction to Nigerians, in whom the association is almost exclusively with **N** and **S**. In the small group of Hira of East Africa there is a similar association with **N** and **S**. In the Cape Coloureds we observed yet a different preference—this time for **M** and **s**.

In the South African Bantu **He** appears to have a random distribution

relative to the M-N-S phenotypes. Table 2 shows a statistical comparison of the 247 **He**-positive bloods (found among 4,000 Bantu tested in this investigation) with 205 unselected Bantu bloods previously reported.[5] The difference between these two samples is not statistically significant.

Table 2: Comparisons of Phenotype Distributions of Two South African Bantu Samples

Phenotypes	*Observed*		*Expected*	
	Random	**He**+	*Random*	**He**+
M.S	20	20	18·1	21·9
M.s	49	70	54·0	65·0
MN.S	25	18	19·5	23·5
MN.s	75	101	79·8	96·2
N.S	7	6	5·9	7·1
N.s	29	32	27·7	33·3
Totals	205	247	205	247

$\chi^2 = 5{\cdot}07$ for 5 d.f. $\quad P > {\cdot}30.$

Similar comparisons were made in respect of the ABO, Rh, Fy^a, K, P and Lewis blood group phenotypes, but no evidence of association was disclosed.

In all, the pedigrees of 24 of the 47 **He**-positive Coloured persons were traced. In 9 cases the pedigrees were too small or too incomplete for analysis, but the remaining 15 yielded excellent material for genetic study. Seven representative examples are illustrated.*

Pedigree A (Fig. 1) shows the **He** factor transmitted in association with **M** and **s** in 3 generations in two kindreds joined by marriage.

Pedigree B (Fig. 2) also shows two kindreds joined by marriage. In the kindred on the right-hand side of the diagram, the **He** factor is clearly associated with **M** and **s** and in the kindred on the left with N and **s**.

Pedigree C (Fig. 3) shows the **He** factor segregating with **M** and **S** in two generations.

Pedigree D (Fig. 4) shows the same association with **M** and **S** unequivocally through three generations.

Pedigree E (Fig. 5) is somewhat more complex. It shows the mating of two **He**-positive persons (I, 4 and 5). The phenotypes are consistent with transmission of *He* in association with *M* and *s* in the mother and with *M* and *S* in the father. In the second generation II, 1, 3, 5 and 6 are presumably homozygous for the *He* gene. Titrations of a potent anti-**He** serum were performed against carefully prepared 2% cell suspensions of each of the siblings of this mating, but no double dose effect could be demonstrated in any of them.

In pedigree F (Fig. 6) both mates are **He**-positive and clearly hetero-

* The complete set of pedigrees was presented as an exhibit at the Sixth International Congress of Blood Transfusion in Boston in September 1956.

zygous since there are two MN **He**-negative siblings. Of the remaining siblings the two who are MN are clearly heterozygous, but it is not possible to determine whether the 4 who are M are homozygous or heterozygous for the *He* gene.

Only one exception to the rule of complete linkage was encountered in this study, viz. pedigree G (Fig. 7). All the siblings of the first generation are **He**-positive, being either M or MN. Several of the mates are dead; but since each of the 6 siblings has had one or more **He**-negative children, all the sibling of the first generation must be heterozygous for the *He* gene. With one exception, the phenotypes of the siblings of the second generation are consistent with the assumption that the *He* gene in this family is being transmitted in association with *M* and *s*. Fig. 7A shows the mating of I, 14 (who is MN, **He**-positive) with her second husband (I, 15) who is group N, **He**-negative. Two of the sibs of this mating are group N, but one (II, 33) is **He**-negative, as expected, while the other (II, 29) is **He**-positive.

The presence of the **He** factor in II, 29 admits of two possibilities: Either I, 15 is not the father of the child, the real father having possessed the *He* gene linked with *N*, or there has been a crossing-over between the genes for the *M-N* and *He* loci of the maternal chromosomes. All the members of this pedigree were repeatedly re-tested and the mother carefully interrogated. The child (II, 29) was born in 1947. The mother (I, 14) has consistently denied that she ever had sexual relations with any man other than her second husband after the death of her first husband in 1940. However, it transpired that she had cohabited with her second husband for two years before she married him in 1948, II, 29 having in fact been born out of wedlock. This was contrary to her original story. The complete phenotypes of the mother, husband and child do not exclude paternity except perhaps in this one particular—the aberrant *He* gene. Nevertheless, it is submitted that illegitimacy must be considered a more likely explanation of the anomalous association of the **He** factor with **N** than crossing-over between the loci for the *M-N* and *He* genes in the maternal chromosomes.

With this solitary exception, all the pedigrees studied in this investigation are consistent with multiple allelomorphism or complete linkage between the genes for *M*, *N*, *S*, *s* and *He*. If the exception is admitted, we are led to the possibility of close but not absolute linkage between the loci for the genes determining the *M-N-S-s* combinations and that for *He*, with crossing-over occurring only occasionally.

In the face of this latter possibility, it becomes necessary to examine the significance of the marked differences observed in the proportional distributions of the M-N-S-s-He phenotypes of the various ethnological groups.

Assuming that the *He* gene is incompletely linked with the *M-N-S-s* genes and that crossing-over occurs—however infrequently—a dispropor-

tion between the M-N-S-s phenotypes containing **He** and those lacking this factor could conceivably occur either by chance or by natural selection. Chance alone could not explain a sustained association of this nature, since the irresistible trend would be towards the re-establishment of genetic equilibrium in succeeding generations; nor is there any evidence to date that the *He* gene alone or in combination with any of the possible *M-N-S-s* genotypes possesses any selective advantage.

No instance of sensitization to the **He** factor has yet been recorded in human beings and the present author has inoculated two **He**-negative volunteers with **He**-positive blood intermittently for over a year without inducing sensitization to this factor. In the case of the West Africans, the *He* gene appears to be associated almost exclusively with *NS* which, of the 4 *M-N-S-s* gene complexes, is by far the most infrequent in the population as a whole. Thus, if linkage is the cause of this disproportion, we have here a situation which makes it necessary to postulate some genetic influence or combination of influences which has not only counteracted the inevitable tendency towards the maintenance of equilibrium, but which has also reversed the process and directed it to the extreme limit of the most unlikely of all the possible associations.

The *NSHe* gene combination is not the only one which has been favoured in this way. In the random sampling of Hottentots, it is *MS* which predominates in association with *He*, while in the South African Bantu there is a slight bias towards an association with *Ms*. From the foregoing, it would therefore appear that the mere existence of statistically significant differences in the proportions of **He**-positive and **He**-negative M-N-S-s types in different ethnic groups is *prima facie* evidence against the possibility of incomplete linkage.

The marked differences in the distribution of the M-N-S-s-He phenotypes in different ethnic groups can, at least to some extent, be explained by the nature of the tribal structure of the African peoples. The number of individual tribes comprising the African population is legion and, especially in North, Central and East Africa, few have escaped the influences of miscegenation with Hamites, Semites, Indians and Europeans at different times in history. The tribes themselves are for the most part patriarchal in structure and, despite the occasional incursions of foreign elements, many have remained socially and genetically isolated through numerous generations. The extent of this tribal isolation may be judged from the fact that in the Province of Bouchi in Northern Nigeria, for example, over 60 different languages are spoken. In these circumstances, polygamy, domestic slavery and inbreeding might well have favoured unusual genetic combinations. This is reflected in the stature and physical characteristics, which are often markedly different in contiguous tribes. Influences such as these have undoubtedly been responsible in large measure for the marked tribal

differences in the incidence of the sickling trait, and could easily account for a disproportionate currency given to one or other of the infrequently occurring genetic factors such as the *NSHe* gene in some tribes. It would, however, be extremely surprising if the almost exclusive association of the **He** factor with **N** and **S** observed in the particular sample of West Africans studied by Chalmers *et al.* should prove to be consistently characteristic of all the tribal groups comprising the West African peoples.

The distribution of the *He* gene in contemporary American negroes, among whom tribal distinctions have disappeared, is readily explained on purely historical grounds. Although a preferential association with *NS* is still apparent, this phenomenon is not nearly as marked as in the Nigerian sample quoted. Most of the negro slaves imported into America were brought from the Gold Coast and its hinterland. Later, some were brought from the Belgian Congo and elsewhere. As has been shown, the small sample of Congolese reported by Nijenhuis[6] differs markedly from Nigerians in the distribution of the M-N-S-s-He phenotypes. This admixture is consistent with the findings in present-day negroes of New York.

The Cape Coloureds in South Africa are also of recent origin and the contributions of Hottentots, Bushmen, Malays, Indians, Bantu and Whites are written large in the diversified physical characteristics of this highly heterogeneous group. Chance alone would have favoured the transmission of the *He* genes associated with *M* from both the Hottentots and the Bantu.

Thus it may be concluded that a theory of completely linked genes is supported by the population data as well as by the direct evidence of inheritance studies. With regard to the question of closely linked *versus* multiple allelomorphic genes, the same arguments apply as in the case of the Rh system, which has similarly been shown to comprise a large number of separately identifiable blood factors. If the individual blood factors and their reciprocals are determined by closely linked genes, the linkage relation is so close that crossing-over practically never occurs. From the genetic point of view distinction between closely linked genes and multiple allelomorphic genes thus becomes purely polemical.

It has been proposed[7] that the letters *L* and *l* be employed to represent the *M* and *N* genes respectively, in honour of Landsteiner who, together with Levine, discovered this blood group system. The gene symbols employed by Wiener for the M-N-S-s system have been expanded to include the rare bloods failing to react with anti-**S**, anti-**s** and anti-**U** sera by using the superscript ^{o} to designate the absence of the genetic determinants for **S**, **s** and **U**. The presence of *He* is indicated by the symbol prime (′) and of *Hu* by double prime (″). When neither is present, the symbol ^{x} has been used, and when both are present in the gene this has been indicated by the superscript ^{y} (Tables 3 and 4).

In accordance with the theory of multiple alleles, 24 genes can be postu-

Table 3: The M-N Genes, Agglutinogens and Blood Factors

Genes	*Agglutinogens*	*Blood Factors Present*						
		M	**N**	**S**	**s**	**U**	**He**	**Hu**
L^{ox}	M	+	−	−	−	−	−	−
$L^{o'}$	M′	+	−	−	−	−	+	−
$L^{o''}$	M″	+	−	−	−	−	−	+
L^{oy}	M^{y}	+	−	−	−	−	+	+
LS^{x}	MS	+	−	+	−	+	−	−
LS'	MS′	+	−	+	−	+	+	−
LS''	MS″	+	−	+	−	+	−	+
LS^{y}	MS^{y}	+	−	+	−	+	+	+
Ls^{x}	Ms	+	−	−	+	+	−	−
Ls'	Ms′	+	−	−	+	+	+	−
Ls''	Ms″	+	−	−	+	+	−	+
Ls^{y}	Ms^{y}	+	−	−	+	+	+	+
$/o^{x}$	N	−	+	−	−	−	−	−
$/o'$	N′	−	+	−	−	−	+	−
$/o''$	N″	−	+	−	−	−	−	+
$/o^{y}$	N^{y}	−	+	−	−	−	+	+
$/S^{x}$	NS	−	+	+	−	+	−	−
$/S'$	NS′	−	+	+	−	+	+	−
$/S''$	NS″	−	+	+	−	+	−	+
$/S^{y}$	NS^{y}	−	+	+	−	+	+	+
$/s^{y}$	Ns	−	+	−	+	+	−	−
$/s'$	Ns′	−	+	−	+	+	+	−
$/s''$	Ns″	−	+	−	+	+	−	+
$/s^{y}$	Ns^{y}	−	+	−	+	+	+	+

Table 4: The M-N Phenotypes

M	**N**	**S**	**s**	**U**	**He**			
					−	+	−	+
+	−	−	−	−	M.	M.He	M.Hu	M.He.Hu
+	−	+	−	+	M.S.	M.S.He	M.S.Hu	M.S.He.Hu
+	−	−	+	+	M.s	M.s.He	M.s.Hu	M.s.He.Hu
+	−	+	+	+	M.Ss	M.Ss.He	M.Ss.Hu	M.Ss.He.Hu
−	+	−	−	−	N.	N.He	N.Hu	N.He.Hu
−	+	+	−	+	N.S	N.S.He	N.S.Hu	N.S.He.Hu
−	+	−	+	+	N.s	N.s.He	N.s.Hu	N.s.He.Hu
−	+	+	+	+	N.Ss	N.Ss.He	N.Ss.Hu	N.Ss.He.Hu
+	+	−	−	−	MN.	MN.He	MN.Hu	MN.He.Hu
+	+	+	−	+	MN.S	MN.S.He	MN.S.Hu	MN.S.He.Hu
+	+	−	+	+	MN.s	MN.s.He	MN.s.Hu	MN.s.He.Hu
+	+	+	+	+	MN.Ss	MN.Ss.He	MN.Ss.Hu	MN.Ss.He.Hu
					−	−	+	+
					Hu			

lated at present. Of these, 8 (i.e. those lacking the genetic determinants for **S**, **s** or **U**) must be extremely rare.

Theoretically, there can be 300 different genotypes and 48 phenotypes can be distinguished, using the 7 antisera for the factors **M**, **N**, **S**, **s**, **U**, **He** and **Hu**. Sanger *et al.*[8] have shown that **U** is absent only when **S** and **s** are lacking in the phenotype. Anti-**U** serum is, therefore, a useful addition in phenotyping, especially since the available anti-**s** sera are apt to give weak and unreliable results—particularly in negroids. Anti-**He** serum is easy to prepare and together with the antisera for **M**, **N**, **S**, **s** and **U** it makes possible the differentiation of 24 phenotypes. Of these, the **U**-negative types are rare and none has so far been found in a series of 600 Bantu bloods tested in our laboratories. Studies of the **Hu** factor are at present seriously handicapped by the rarity of the corresponding antiserum. This is unfortunate as the **Hu** factor has a fairly high frequency in Africans and by its addition the number of distinguishable phenotypes is increased from 24 to 48.

The frequencies of the 12 *M-N-S-s-He* genes in the South African Bantu shown in Table 5 have been calculated by correlating (i) the observations of the M-N-S phenotypes of the 247 **He**-positives found in the series of 4,000 random bloods with (ii) the M-N distribution found in another series of 1,115 random Bantu bloods and (iii) the M-N-S phenotype distribution found in the series of 205 bloods previously reported.[5]

Table 5: Calculated Gene Frequencies in South African Bantu

Henshaw-Negative		*Henshaw-Positive*	
Genes	*Frequencies*	*Genes*	*Frequencies*
L^o	0·0000	$L^{o'}$	0·0000
LS	0·0857	LS'	0·0015
Ls	0·4420	Ls'	0·0193
l^o	0·0000	$l^{o'}$	0·0000
lS	0·0465	lS'	0·0006
ls	0·3944	ls'	0·0099
	0·9686		0·0314

Summary

The Henshaw (**He**) blood factor has been investigated in Whites, Cape Malays, Cape Coloureds and Bantu in South Africa. It is absent or rare in Whites. In Cape Malays it has a frequency of 2%, in Cape Coloureds 4·7% and in Bantu 6·2%.

The association of the **He** factor with the **M-N-S-s** factors in the phenotypes differs markedly in certain ethnic groups. For example, in Nigerians

the association appears to be almost exclusively with **N** and **S**; in Hottentots and Congolese, it is predominantly with **M** and **S**; in the South African Bantu it has a fairly random distribution; while in the Cape Coloureds it is predominantly associated with **M** and **s**.

The inheritance of the **He** factor has been studied in a number of Coloured pedigrees. With only one exception, the segregation of the genes was compatible with complete linkage between the *He* gene and the *M-N-S-s* genes.

The possibility that this one exception might be due to crossing-over is discussed and it is concluded that illegitimacy is a more likely explanation.

The genetic significance of the variability in the association of **He** and the various M-N-S-s phenotypes in different ethnic groups is discussed and it is concluded that this, too, favours the theory of complete linkage or multiple allelomorphism, since incomplete linkage would inevitably tend towards the establishment of equilibrium in the proportional distribution of the **He** factor relative to the various M-N-S-s phenotype combinations.

The available evidence indicates that the Hunter (**Hu**) factor is related to the M-N-S system in the same way as is the factor **He**.

The multiplicity of blood group factors, agglutinogens and genes comprising the M-N system thus provides a genetic situation entirely analogous to the Rh-Hr system.

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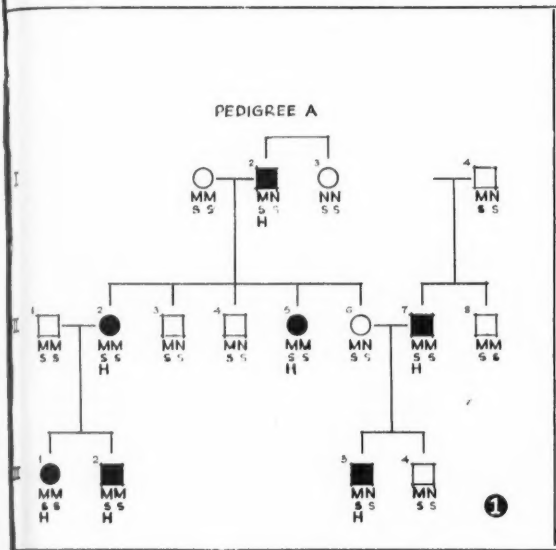
PEDIGREE A
I
II
III
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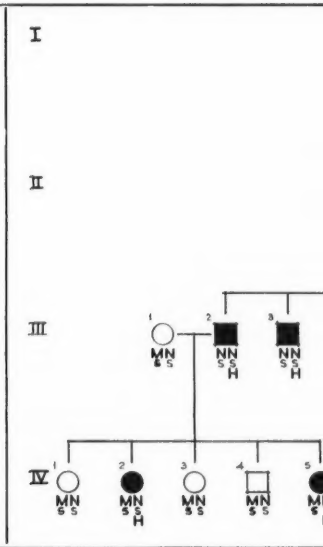
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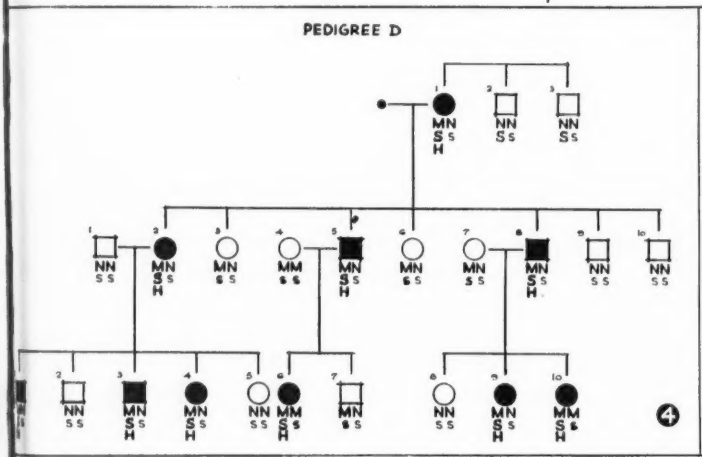
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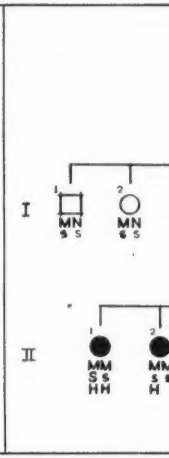
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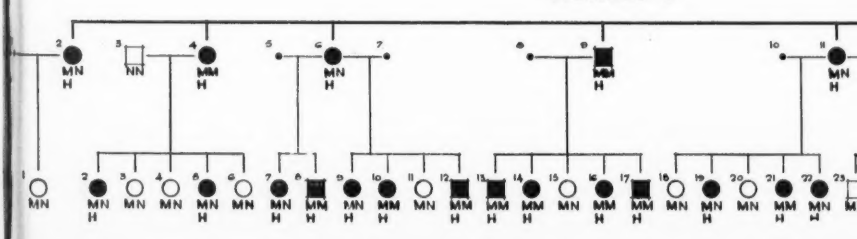
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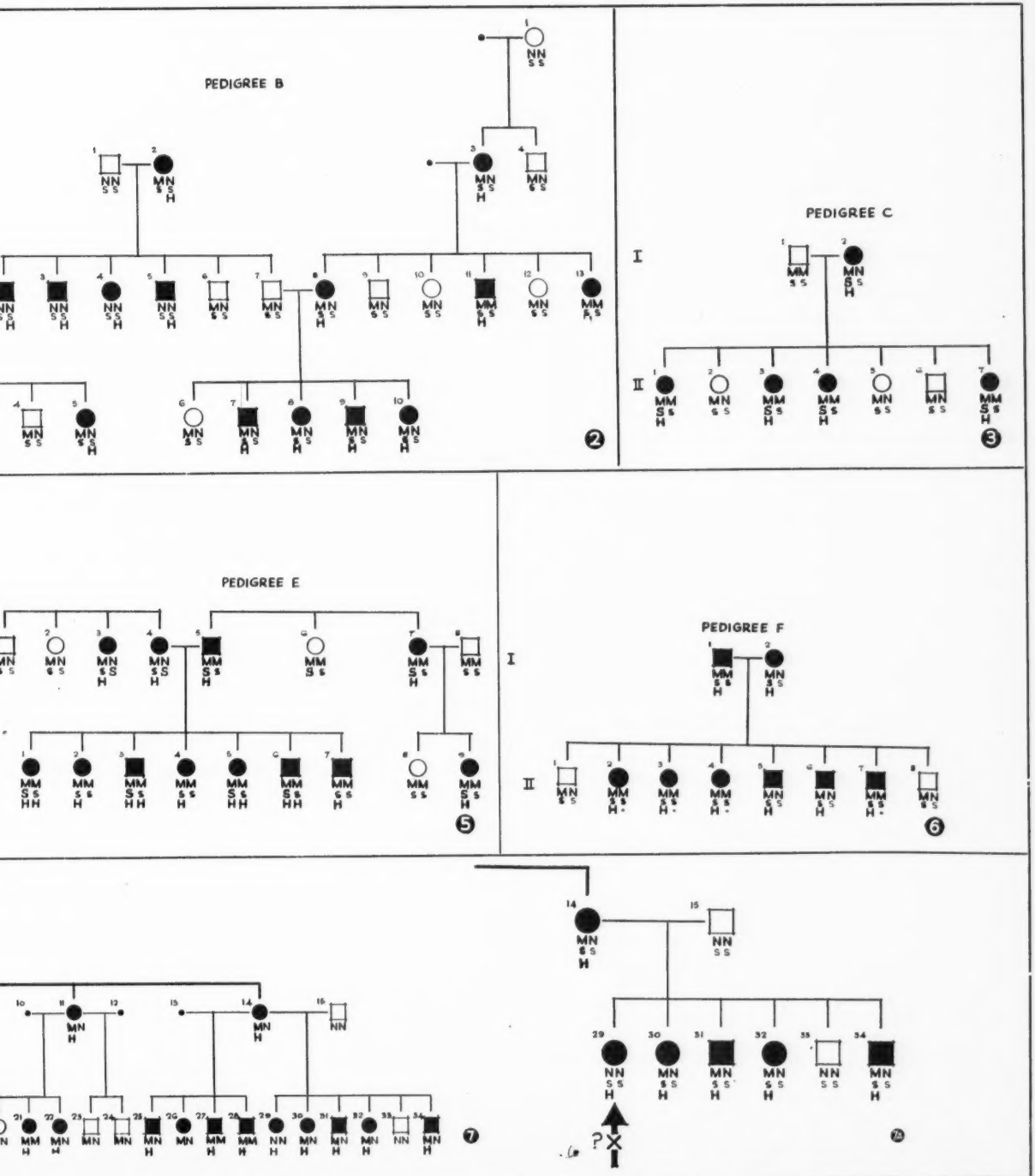

To face p. 160

Post-Mortem Persistence of Sex Chromatin

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and

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A morphological distinction between the nuclei of intermitotic nerve cells of male and female cats was described by Barr and Bertram (1949), viz. the presence in the female nerve cell of an intranuclear mass of chromatin lying adjacent to the nucleolar membrane. The distinction was later shown to be present in the cells of other tissues in the cat (Graham and Barr, 1952), and also in embryonic tissues in the same animal (Graham, 1954).

Moore, Graham and Barr (1953) demonstrated this sex difference in the nuclei of cells of the epidermis of human females, a finding which was extended by Moore and Barr (1954) who observed that the dissimilarity in nuclear morphology between the sexes appeared to be a phenomenon in human tissues and organs which has few exceptions. The intranuclear mass of chromatin was originally called the nucleolar satellite but as its position is variable, for example in the cells of many tissues it is adjacent to the nuclear membrane, the term which implied its topography was discarded and it was renamed the sex chromatin which explained its probable importance.

The application of these findings, in the form of the skin biopsy method of sex determination, has proved of clinical value in the diagnosis of various types of abnormal sex development. More recently the simpler method of examining smears of oral epithelial cells has proved to be equally efficacious and is likely to eliminate the necessity for skin biopsy (Moore and Barr, 1955; Dixon and Torr, 1956).

In addition to their clinical value, it is probable that these methods might usefully be employed in medico-legal cases to aid, for example, in the identification of a body mutilated beyond recognition; for if post-mortem changes are not too advanced, it may be possible to determine the sex of the individual from a small fragment of tissue. However, the length of time after death during which the sex chromatin may be expected to remain as a readily demonstrable entity has not yet been established and the present investigation has been undertaken for this reason.

The persistence of this nuclear feature was studied in dissecting room material and in the skin of full-term human foetuses, exemplifying respectively fixed and unfixed tissues.

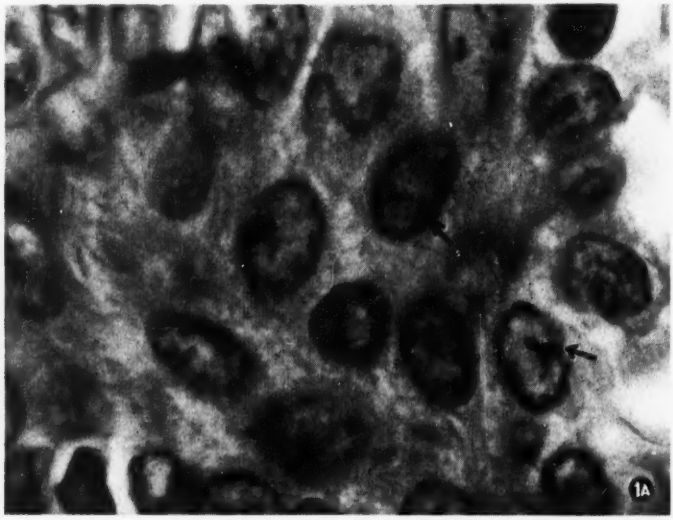
Fixed Tissues

Small pieces of tissue were removed from the head region of 5 dissecting room subjects. They had been completely dissected during the course of the normal teaching programme. No special precautions had been taken to ensure more than routine fixation and, at the time the tissue was removed,

it was impossible to identify the sex of the cadavers other than by the identification labels. Identification of sex from scrapings of the oral epithelium was attempted but, as was anticipated, this did not prove satisfactory. Blocks of tissue were removed from the skin of the face, including portions from the external ear, the tongue and the mucoperiosteum of the hard palate. They were placed in 10% formol-saline for 24 hours before they were embedded in paraffin wax. Sections were cut at 6μ and stained with Harris' haematoxylin and eosin. The ages of the cadavers ranged from 60—78 years and preservation procedures had begun 2—5 days after death, some 10—12 months before.

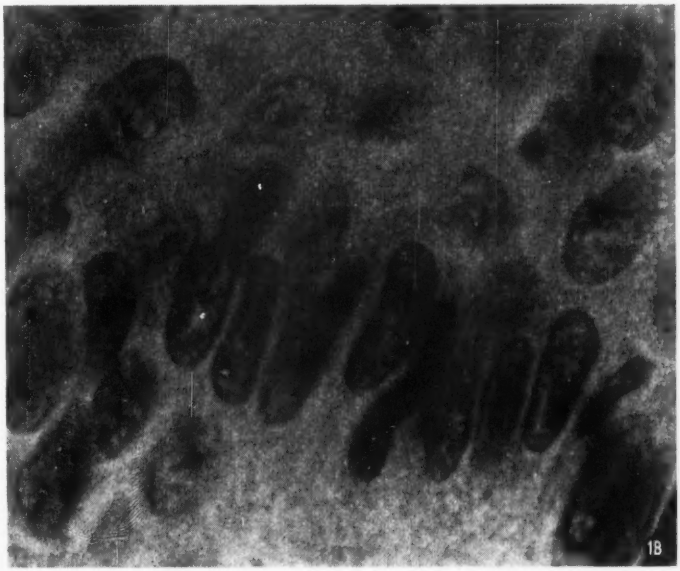
Careful examination under an oil immersion objective showed that the sex of the individuals (of whom 5 were female and 2 were male) could be correctly diagnosed from the various tissues, although in the case of 2 subjects, because of the rather poor state of cell fixation, it was necessary to examine cells from more than one region to confirm the result.

Several nuclei from the basal layers of the epidermis of a female cadaver, aged 60 years at time of death, are shown in Fig. 1A. In these nuclei many

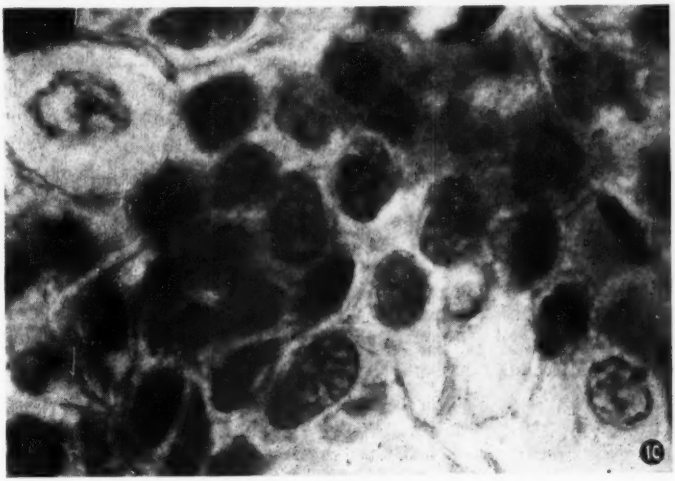


chromatin granules and flakes can be seen, but in those indicated by arrows a much larger mass of more darkly stained material, the sex chromatin, is visible closely applied to the nuclear membrane. The upper of the 2 nuclei which are marked shows the typical plano-convex form of sex chromatin, which is also present in the other nucleus, but is on a slightly different plane of focus. It will be appreciated that it is extremely difficult to demonstrate more than one sex chromatin mass clearly in a single

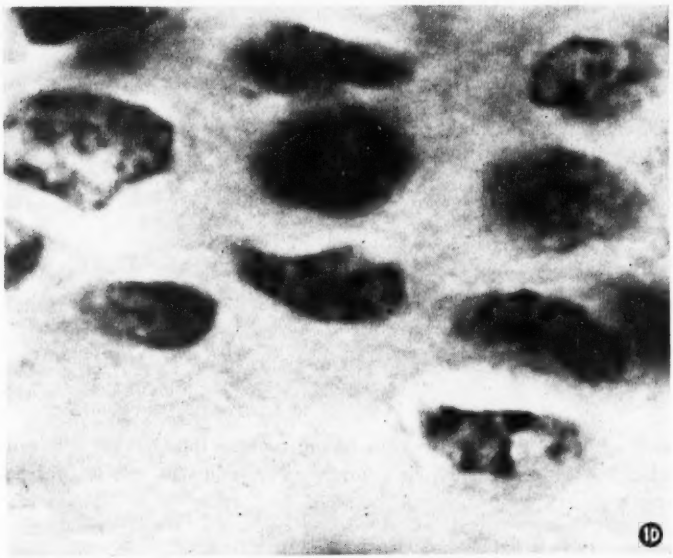
photograph, due to the high magnifications being used and the consequent reduction in the depth of field. Fig. 1B shows nuclei from the skin of a male



subject, aged 70 years. In contrast to the previous photograph, while it is possible to see many chromatin masses in the nuclei, no large bodies, indicative of sex chromatin, can be seen.



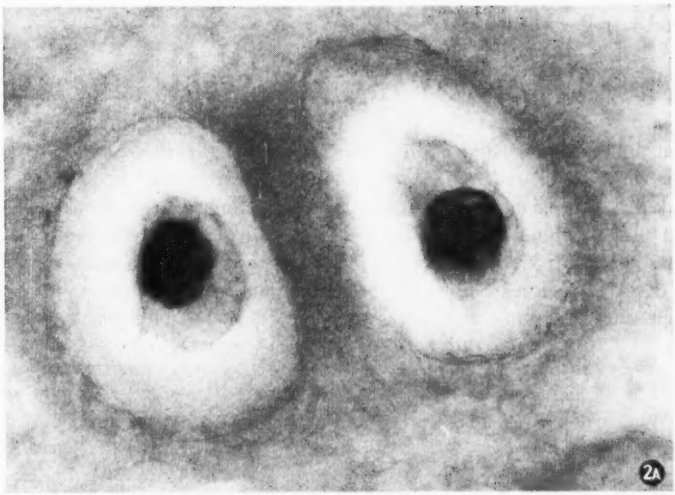
The distinction between the sexes in the disposition of chromatin material in skin also exists in other tissues. Fig. 1C shows examples of nuclei from the mucoperiosteum of the hard palate (female). As before, these are basal cells from the epithelium, for a greater proportion of these show sex chromatin than is the case with nuclei of more superficial layers. Likewise in the mucous membrane of the tongue from the same individual (Fig. 1D) several cells typical of the female are evident.



The pieces of tissue removed from the external ear included small portions of fibro-elastic cartilage and it was noticed as soon as a small amount of material had been examined that sex chromatin in this tissue was more distinct than in the epithelia. An example from a female aged 78 years is shown in Fig. 2A. Sex chromatin is prominent at the nuclear membrane in the nucleus towards the left of the photograph. As the diagnosis of sex from an examination of the morphological features of epithelial cells from this subject was extremely difficult, this observation has considerable significance and may be explained as follows:

The amorphous cartilaginous matrix, being less permeable, epecially after fixation, than that of tissues with an essentially collagenous basis, hinders loss of certain cellular constituents so that they retain more normal histological detail.

This hypothesis was tested by removing pieces of external ear from 2 teaching specimens, which had been preserved by periodic immersion in a

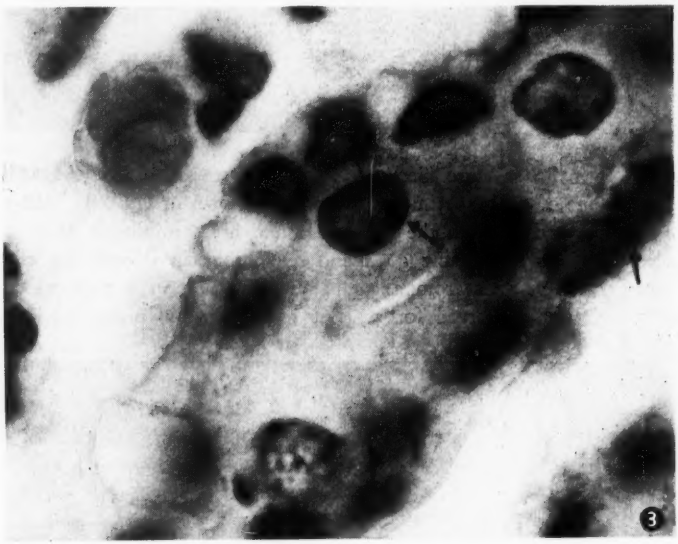


glycerin-formalin mixture. Sections were prepared as before. A cartilage cell from one of these specimens, a female, is shown in Fig. 2B. Although the material had been in the preserved state for a minimum of 5 years, nuclear detail is remarkably good and sex chromatin is well defined. By contrast, cartilage cells from a comparable situation in a male specimen which had been preserved for at least 7 years, while presenting equally

good histological appearances, did not possess sex chromatin.

Unfixed Tissues

To study the persistence of sex chromatin in unfixed tissues, the limbs of a full-term female foetus were exposed to various environments to simulate conditions under which such material might possibly be found. One limb was placed in fresh water, another in the open air, a third in a dark and rather damp cellar and the remaining limb was wrapped in a cloth and placed behind a central heating radiator to encourage mummification. Small pieces of skin were removed at regular intervals, and fixed and stained as described before. The histological appearance of tissue removed at the beginning of the investigation is shown in Fig. 3. Well-defined masses of



sex chromatin are seen in the positions indicated by the arrows. As in the dissecting room material, nuclei of cells of the skin of a male foetus, of comparable post-mortem age, did not possess typical masses of sex chromatin.

After several days sex chromatin could still be readily distinguished in tissue kept in all 4 environments and it continued to be visible until about the fifteenth day after death, first disappearing in mummified tissue, but persisting for a longer period in the case of the limb which had been in water. In skin from this limb sex chromatin was observed until the 23rd day, but thereafter, as had been the case with the other limbs from the 15th day, autolytic changes made further accurate histological assessment impos-

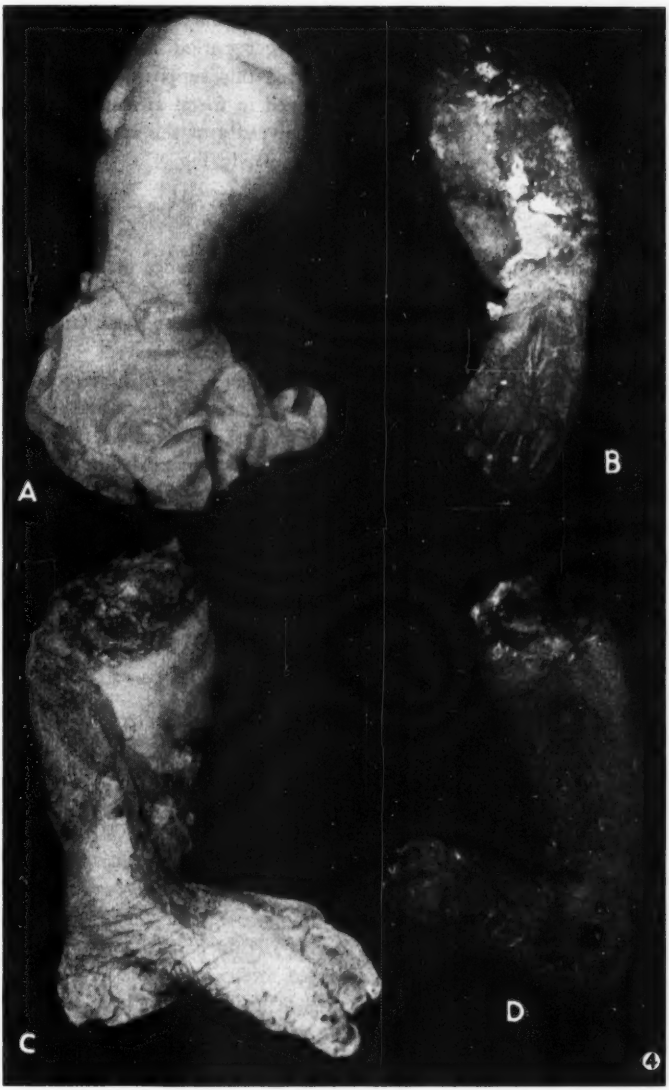


Fig. 4. The foetal limbs at the end of the investigation.
A. Limb which had been in water.
B. Limb which had been in cellar.
C. Limb which had been in open air.
D. Limb which had been behind radiator.

sible. In view of our findings in the case of fixed tissue, it was thought that the examination of cartilage cells might enable the sex of the tissues to be successfully determined after a longer period had elapsed, but a preliminary investigation has not supported this supposition for autolytic changes, with pyknosis of the nuclei, proved in foetal articular cartilage to be as extensive as in other tissues. The diversity of the appearance of the limbs at the end of the investigation is shown in Fig. 4.

Conclusions

From this investigation into the post-mortem persistence of sex chromatin certain conclusions emerge:

1. The sex of small fragments of fixed epithelia and cartilage can be determined by an examination of nuclear morphology.

The length of time for which the tissues have been preserved is not significant, sex differences having been observed in tissue which had been in the preserved state for periods up to 7 years.

2. By employing a similar technique it is possible to determine the sex of unfixed tissues, but in this case the period during which accurate diagnosis may be made is very much shorter. It varies from 2—3 weeks after death in foetal tissues, depending on the nature of the environment in which the tissue has been placed.

3. In the application of these findings to the determination of the sex of an individual in medico-legal cases, cartilage cells should, if possible, be obtained for examination, as they appear to retain the normal morphological features of sex chromatin to best advantage.

We are most grateful to Prof. G. A. G. Mitchell for his permission to undertake this investigation in the Department of Anatomy and for his continued advice and encouragement.

Our thanks are also due to Mr. P. Howarth, A.R.P.S., who was responsible for the photography.

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Identification by Patterns

Paper Separation Methods

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Determination of the identity of source of 2 items of evidence is probably the most important and frequent task confronting the criminalist. Throughout the entire area of source identification, the matching of patterns of an unknown with a known is by far the most satisfactory and definite method. This is true of fingerprints, spectrograms, absorption spectral curves, X-ray diffraction patterns, bullet and tool mark comparisons by the comparison microscope, and other widely used types of procedure. The unique value of the photograph in the study and comparison of evidence results largely from this very necessity of comparing patterns.

The choice of the type of pattern to be compared will be determined by:

(*a*) The availability of equipment, which is often subject to high cost or complexity;

(*b*) Richness of detail of the pattern which determines the reliability of the identification; and

(*c*) Several secondary considerations such as necessary training, speed of operation, size of sample necessary, and destruction or preservation of the sample.

It is clear that the ideal method of carrying out such identifications would be one that provides maximum detail in the pattern, very minute amounts of material which is not destroyed or altered in the comparison process, and simplicity of equipment and technique combined with suitable speed of operation. This is a difficult if not impossible combination which is best approached by the comparison microscope, which (when applicable) meets most of the requirements but suffers from considerable subjectivity and limited application. The spectrograph also is limited in application, requires a significant size of sample, and destroys the evidence. The equipment is expensive as is also the X-ray diffraction method, which likewise has serious limitations of application, and requires specialized training of a high order.

In recent years a new type of pattern has been developing in several fields of science, both theoretical and applied, viz. the patterns obtained on paper

by chromatography or by zone electrophoresis. It is the purpose of this paper to present and illustrate some favorable techniques and equipment in this field and to discuss the applications and value of the methods in criminalistics, in the identification of physical evidence as to type and source.

Paper Chromatography

Paper chromatography,[1-4] developed first by Martin and Synge[5] and others, was so startling in its simplicity that it almost immediately captured the attention of biochemists and other research scientists. Most laboratory workers had observed it in a simple form when they added a solution of colored material to filter paper in their ordinary operations, but the full significance had not been appreciated. Developed chiefly for the separation and identification of amino acids, it was soon apparent that its applications were very general and it is now possible to resolve almost every type of soluble mixture. Perhaps its best known applications in criminalistics have been in the identification of ink[6-9] in which the preformed mixture of colored materials requires no separate identification after separation of the constituents. Further, the ink constituents may be separated partially by water alone as a developing solvent,[6] though this is a crude and not completely satisfactory technique. The question of the best technique for carrying out the separation has been the chief subject of difference of opinion, since most investigators have standardized on some mixture of butyl alcohol, acetic acid and water, or butyl alcohol, ammonia and water, as the best solvent so far proposed. Ascending strip chromatography is most widely used because of its great simplicity and the fact that the R_f (ratio of the distance through which a material moves to the distance the solvent moves in the same time) values of ink constituents are generally high, so that good resolution is reached without prolonged solvent passage. For the same reason, descending strip methods are not considered especially suitable and considerably more difficult in instrumentation. This author prefers the horizontal method because it is fully as simple as ascending methods; it allows direct comparison of more samples than are convenient by the ascending strip; and it is faster. It can be advantageously substituted for ascending strip methods whenever the latter are useful.

The technique of horizontal chromatography is essentially that of Giri and coworkers.[10, 11] Two flat vessels, such as glass pie plates, are ground flat on the rim so as to fit like a desiccator lid and body. In the center is placed a vial or short length of large glass tubing, in a vertical position. Circular filter paper (Whatman #1 is satisfactory), slightly larger than the vessels, is drilled at the center with a large cork borer slightly larger than the vertical glass tube. Around this hole is ruled with a pencil compass a concentric ring about $\frac{1}{4}$–$\frac{1}{2}$ inch from its periphery. On this ring are placed the samples in a circle, far enough apart that no admixture of samples

occurs. From 6–8 samples may be placed on a single paper. A strip of filter paper is wrapped around the glass central tube to serve as a wick, and the filter sheet is pushed over this wick. The solvent mixture is placed in the bottom of the lower vessel, and the wick and paper are inserted. Finally, the top vessel is put in place to seal the system against evaporation.

Ink is separated into its constituents in 5 to 35 minutes, and the resulting fan-shaped chromatograms on the same paper may be compared directly. The method has been shown to be highly favorable for liquid writing inks[9] and uniquely so for ball point pen inks[8] which are not readily studied by other methods. It is being used for study of chemical changes in ink as it ages, and for a variety of identification problems not related to ink.

Perhaps the most extensive use of paper chromatography in the field of criminalistics has been in the study of drugs and poisons. This has been recently reviewed by the author.[12] Here the concern has been far less with pattern identification than in separating and studying mixtures such as alkaloids from various species, though some direct identification of poisons and narcotics has been described.[13] These scarcely fall in the field of pattern identification which is concerned with an over-all identity rather than the presence of a single constituent. Paper chromatography in inorganic analysis has been extensively studied,[14-16] but little applied in criminalistic practice. There appears no reason why it should not be valuable in this field.

Paper Electrophoresis

Electrophoretic separations of complex mixtures on filter paper have been widely utilized for protein and enzyme fractionation because materials of this type are often denatured by chromatographic solvents. For such substances, paper electrophoresis has proven extremely effective and is now a standard clinical method for analysis of the serum proteins. Movement in an electrical field is characteristic of any charged particle, large or small. The charge may be on an ion, or it can result from surface effects in the case of colloidal micelles and even larger particles. Since so many of the materials of interest to the criminalist are ionic, the production of characteristic patterns on paper by differential mobility of the constituents is an obvious method of utilizing patterns for identification purposes. It has been used for identification of ink,[9] in which it was very successful. An illustrative pattern is shown in Fig. 1. It also has received some preliminary use in the field of drugs and poisons,[17-19] and in inorganic analysis.[20-23]

Although the chief use of paper electrophoresis has been in the study of blood, no applications of the technique in medico-legal blood problems appear to have been made. It has been noted[24] that conventional electrophoretic serum protein patterns show a degree of individuality which may assist in the ultimate identification of the individual origin of blood. Similar findings have also been indicated as a result of paper electrophoretic analysis of blood proteins. This would appear to be a very limited approach to this

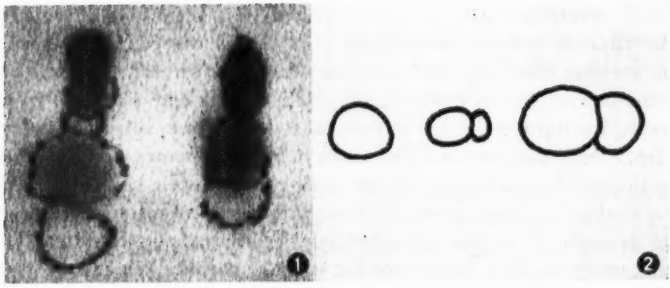

Fig. 1. Electrophoretic Patterns: Two common washable black inks.
Fig. 2. Electrophoretic Pattern: Metals from hypodermic needle wire (tracing).

very important problem; but to the extent that such patterns might differentiate between the blood of 2 or more persons, it would have immediate value in the field of forensic medicine. Since most of the blood encountered by the investigator is in the form of dried spots or areas, any such application would be more valuable if applied to dried blood than to fresh serum or plasma. No record is found in the literature of any efforts in this direction. In a later section, some preliminary results of a modified technique will be discussed.

Because of the early emphasis on the clinical applications of electrophoresis in the study of proteins, the very obvious applications to criminalistic problems involving separation and identification of small ions such as those of the metals appear to have been largely overlooked. Since metals figure frequently in crime investigation in the form of various alloys, paint and other industrial products, and in toxicology, this oversight is unfortunate. Separation of metal ion species by electrophoresis on paper[25] has been studied extensively in the author's laboratory, and it is established that most of the metals of interest to the crime investigator may be isolated on paper, and detected in very minute quantities, i.e. fractions of a microgram. When the samples are especially small, as in testing tiny fragments of metals and alloys, paint and similar material, the advantages of electrophoresis over such conventional methods as spectrography become apparent. They are as fast, they utilize considerably smaller quantities and, with metals and alloys, it is possible to use a virtually non-destructive procedure with replicate analysis. Fig 2 shows a typical paper separation of the metals making up the wire from a No. 20 hypodermic needle. An imperceptible amount was dissolved from the end by an electrosolution method[25] and this was separated into the 4 areas illustrated, by electrophoresis on paper.

Electrochromatophoresis

Some of the most detailed patterns, and therefore the most reliable ones for identification purposes, have been obtained by use of a combination of

paper electrophoresis with a device that allows solvent flow at approximate right angles to the direction of the electric current. In some systems there is considerable adsorption on the paper fibers, so that separation is obtained by flow of an aqueous buffer only. When current is passed at right angles, a 2-dimensional separation results without an increase in the time or effort of the analysis. The equipment illustrated in Fig. 3* and its method of use

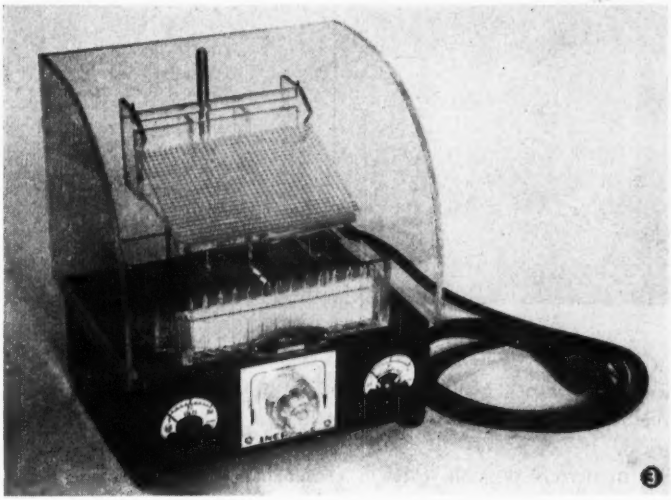

Fig. 3. Microapparatus for electrochromatophoresis.

were described by Karler, Brown and Kirk.[26] The apparatus is small, weighing less than 4 lb. and measuring only 8 inches in its greatest dimension, yet it produces separations comparable with those of the very large and expensive continuous electrophoresis apparatus. It works on the same principle as the latter type of equipment, but is highly advantageous for analytical uses as well as for continuous separations. The required sample is much smaller than that for any conventional electrophoresis equipment now on the market, being limited only by the limits of identification of the separated components, which is usually in the sub-microgram range.

A previously unpublished modification of this equipment, in which 2 samples are separated simultaneously, is shown in Fig. 4. The design is advantageous for criminalistic purposes because it allows 2 samples that are to be compared to be analyzed under identical conditions. It has 2 separation areas of the same dimensions and fed with buffer from the same

* Obtainable from the Microchemical Specialties Co., Berkeley, California.

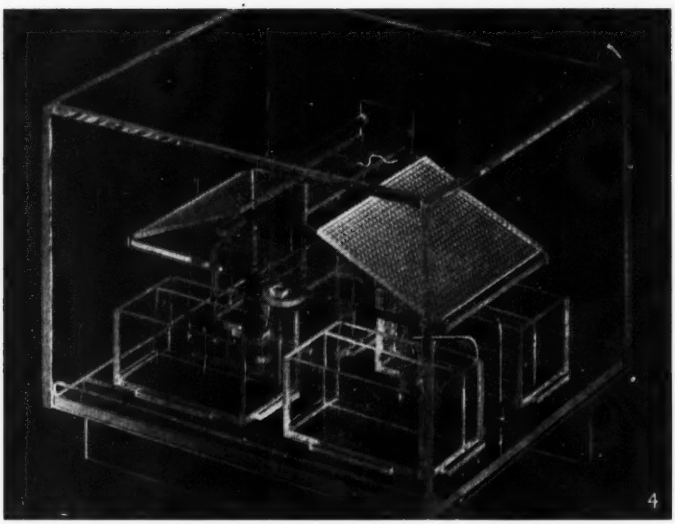

Fig. 4. Double unit microapparatus for electrochromatophoresis.

reservoir. Electrical connexions to the 2 sides of the apparatus are in parallel, so that both the voltage applied and the time of application are identical.

The numerous possible uses in criminalistics of the small, versatile apparatus for electrochromatophoresis have been only partially explored. It has been shown to be very effective in the identification and study of ink[27] and it can also be used for separation and identification of metals.[25] Perhaps the most promising application is in the study of dried blood, with a view to distinguishing between blood from different persons by means that are independent of the blood groups. Preliminary studies indicate that dried blood not only gives a pattern very different from fresh blood, but that bloods with different origins are often different enough in their patterns so that they can be differentiated. Too few data are available at this time to evaluate the method from this standpoint, and the separation of protein fractions achieved at present is less satisfactory than is desirable because of the extensive denaturation that occurs on drying of the blood proteins. More extensive studies in this direction are urgently needed because of the strong probability that bloods of different individual origins may ultimately be distinguishable in all or nearly all instances as techniques are perfected. A typical pattern of dry blood is shown in Fig. 5.

Similar considerations may well apply to physiological fluids other than blood, but no data concerning such applications are available. It is of interest in this connexion that urine has been studied in a limited fashion

with a view to the characteristic personal differences that it may show.[28] Additional methods of personal identification must remain a major goal of criminalistic research, and it appears that the patterns produced in various paper separation methods provide an important step toward that goal.

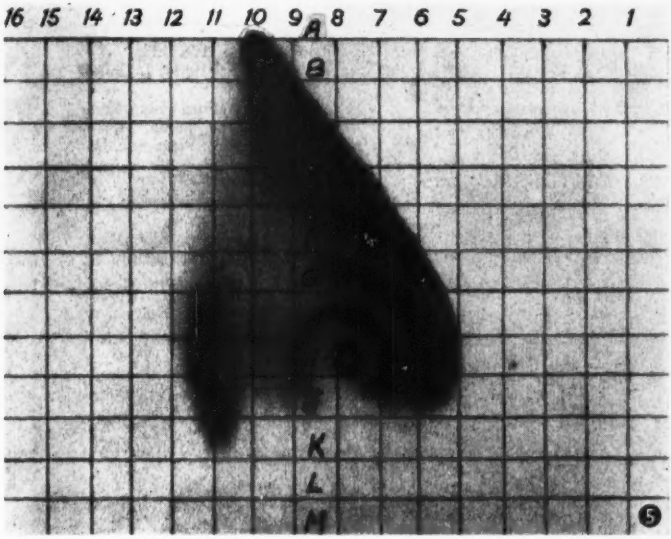

Fig. 5. Electrochromatophoretic pattern of typical dried blood.

Summary

In paper chromatography and paper electrophoresis, the crime investigator possesses a type of tool by which identification through the production of characteristic patterns may be greatly extended.

Some techniques and applications of this approach to the identification and possible individualization of blood, metals, inks and other materials are discussed.

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